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Award Number: W81XWH-10-1-0467

TITLE: Prevention of Premalignant Progression of Human Breast Cancer.

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REPORT DATE: August 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
August 2011	Annual	15 July 2010 – 14 July 2011

4. TITLE AND SUBTITLE

Prevention of Premalignant Progression of Human Breast Cancer.

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-0467

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Baylor College of Medicine
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The main aim of these experiments is to test the effect of selected drugs on premalignant progression of a defined set of human breast premalignant cell lines using a newly developed intraductal xenograft model. In the first phase of these experiments, we used three cell lines, two of which show different background rates of progression to invasive ductal beast cancer (DCIS.com and CCH1.dcis) and a third line, SUM225, which does not exhibit local invasion. The basic protocol involves intraductal injection of cells into primary duct of immunocompromised hosts, followed by a 4-6 week treatment of agents beginning at 6 weeks after intraductal injection. The endpoints are extent of growth in vivo, Qt-PCR and IHC of biomarkers specific for the chemopreventive drug of interest. The initial experiments involve a 3 x 3 design (three cell lines and three agents). Six groups have been set up. The initial results indicate that the protocol is effective and will provide information on the chemopreventive efficacy of specific drugs. The initial data suggest the efficacy is a function of the molecular alterations in the premalignant cell line and the specific target of the chemopreventive agent.

15. SUBJECT TERMS
uman breast, premalignant, progression, xenograft, drugs

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	6	19b. TELEPHONE NUMBER *(include area code)*

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Introduction:

The main aim of these experiments is to test the effect of selected drugs on premalignant progression of a defined set of human breast premalignant cell lines using a newly developed intraductal xenograft model. Three premalignant (DCIS) human breast cell lines were treated with either rexinoid 268, lapatanib, or dasatanib for a period of 6 weeks. The effects of these drugs on in vivo intraductal growth and on specific biomarkers were measured using multiple assays. The overall goal is to develop a simple and rapid xenograft-based assay to test the efficacy of chemopreventive drugs on premalignant progression in human ductal carcinoma in situ.

Body:

The focus of the experiments in year one has been on aim 1. Aim 1 was to determine the responsiveness of DCIS cell lines to targeted agents. For this aim, we originally proposed to examine three cell lines each with three drugs.

Statement of work 1a. To test the efficacy of drugs on growth and progression of DCIS. In year 1, we set up six sets of xenografts which included the following cell lines and targeted agents: DCIS.com treated with rex 268 and dasatanib, SUM225 treated with rexinoid 268 and lapatanib and CCH1.dcis treated with rexinoid 268 and dasatanib. Table 1 shows the effects of these experiments so far. The most marked effect is with dasatanib on progression of CCH1.dcis. With just 4 weeks of treatment, the drug inhibited progression from DCIS to IBC by 40% at the dose used. The effect was manifested by both a decrease in the number and size of the invasive lesions. The experiment with DCIS.com cell line is in progress. The rexinoid 268 did not exhibit any effects on invasion in any of the three cell lines. Lapatanib had a slight inhibition on extent of growth of SUM225. As SUM225 does not invade under these conditions, there was no discernible effect on progression.

Table 1. Summary of cell line and drug effects

Cell line	Drug	In vivo growth	Progression
DCIS.com	rex268	no effect	no effect
DCIS.com	dasatanib	i.p.[a]	i.p.
DCIS.com	lapatanib	year 2	--[b]
SUM225	rex268	no effect	no effect
SUM225	lapatanib	inhibition	--
SUM225	dasatanib	year 2	--
CCH1.dcis	rex268	no effect	no effect
CCH1.dcis	dasatanib	inhibition	inhibition
CCH1.dcis	lapatanib	year 2	--

[a] i.p. = in progress
[b] -- = not applicable

For year 2, we will finish setting up the cell line drug combinations. Depending on the results, we will increase the dose of dasatanib to try to achieve a more marked inhibition. As rexinoid 268 did not have a significant effect on the invasive phenotype in any of the three cell lines, we will examine a fourth agent, metformin. Metformin has received recent interest as a drug which affects growth of breast cancers.

Statement of work 1b. Examine effect of drugs on biomarkers of growth and targeted responses. We measured RNA expression in the human DCIS cells by harvesting whole mammary gland that had been injected with Sum225 cells, preparing enriched epithelial cells, extracting RNA, and then performing quantitative RT-PCR analysis. Typically, 25μg to 50μg RNA was isolated from each sample, and 50ng to 100ng of RNA was used for cDNA and synthesis and quantitative RT-PCR experiments. Standard curves for the quantification of each transcript and cyclophilin (used for normalization) were generated using the serially diluted solution of synthetic templates. Statistical significance was determined comparing the means of triplicate samples using the Student *t* test. All reactions were performed in triplicate.

The expression of six genes was examined after 12 weeks with these preventive agents. These genes include two house keeping genes (cyclophilin and β-actin); proliferation markers (*Ki67* and *cyclin D1*); and rexinoid-regulated genes (Adenosine triphosphate binding cassette transporter A1 (*ABCA1*), and Adenosine triphosphate binding cassette transporter (*ABCG1*)). We are currently measuring the expression of another rexinoid-regulated gene, insulin-like growth factor-binding protein-6 (*IGFBP6*). Our results showed that LG100268 treatment caused reduced expression of *cyclin D1*, but not *Ki67*, while lapatanib treatment caused a reduction of *Ki67* but not *cyclin D1*. LG100268 also induced expression of the rexinoid-regulated genes in the Sum225 cells. Expression of *ABCA1* was significantly increased in rexinoid treated samples (11 fold) compared to vehicle treated samples as determined by QRT-PCR. As expected, the expression of *ABCA1* was unchanged in lapatanib-treated samples compared to vehicle-treated samples. These results demonstrate that both LG100268 and Lapatanib reduce proliferation of the Sum225 DCIS cells, but that they likely suppress proliferation through different mechanisms. We are currently studying other biomarkers in these samples and also are conducting parallel studies with the other preventive agents. These initial experiments demonstrated the feasibility of detecting drug-specific induced changes in human genes in this xenograft system. Additionally, it demonstrated the importance of defining unique sets of genes to examine for each drug as well as examining genes related to general cell function (i.e., cell proliferation).

Key research accomplishments:

- Demonstrated the reliability and usefulness of the xenograft model for human DCIS.
- Demonstrated that one of the three drugs has an effect on growth and more importantly progression in at least one of the cell lines.
- Demonstrated the ability to detect and measure changes in human biomarkers specific for each targeted drug in the DCIS cell lines in an in vivo setting.

Reportable outcomes:

As of this summary, we are only 10 months into active experimentation. At this time, there have been no Abstracts submitted or manuscripts written describing the results.

Conclusion:
In the initial experiments, we have used a unique model of human DCIS to identify and test targeted agents for the property of effectively preventing progression of human ER-negative DCIS into invasive breast cancer. We hypothesize that distinct types of DCIS, as defined at the genetic and cytological levels, will require specific targeted agents to block progression to invasive cancer. It is possible (although highly unlikely, according to our hypothesis) that all subsets will demonstrate the same sensitivity to a given targeted agent. The initial experiments support the feasibility of the xenograft model to identify unique agents to block premalignant progression of human DCIS.

References: None

Appendices: None